Rejection under 35 U.S.C. §102(a) over Baca et al.

Claims 34-38 are rejected under 35 U.S.C. 102(a) as allegedly being anticipated by Baca et al. (1997) *J. Biol. Chem.* 272:10678-10684 (published April 18, 1997; "Baca et al.").

Applicants respectfully submit that the Baca et al. reference describes the inventors' own work and thus should not be deemed as a prior art under 35 U.S.C. §102(a). *In re Katz* 215 USPQ14 (CCPA 1981). Baca et al. was cited apparently because the authorship of the reference and the inventorship of the present application are not the same. Applicants submit herewith a Declaration under 37 C.F.R. §1.132 by co-inventor Dr. Leonard G. Presta, attesting that the subject matter in the Baca et al. reference was derived and produced solely by the conception and design of the named inventors of the above-identified application. The Declaration demonstrates that Shane J. O'Connor, the only co-author of the Baca et al. reference who is not a co-inventor of the present application, carried out some of the experiments reported in the reference under the direct supervision, control and direction of Dr. Presta and or the other co-inventors of the present invention. Since Shane J. O'Connor did not contribute to the conception or design relating to the studies reported in the Baca et al. reference, the subject matter disclosed in the reference as it relates to the present invention is not "the work of another," but the work of the named inventors. Accordingly, applicants submit that the Presta Declaration effectively removes the cited Baca et al. reference as an prior art under 35 U.S.C. §102(a), and respectfully request the rejection be withdrawn.

Rejections under 35 U.S.C. §103(a)

Claims 34-38 are rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Ferrara et al. WO 94/10202 ("Ferrara et al."), and further in view of Adair et al. WO91/09967 ("Adair et al.") and Yelton et al. (1995) *J. Immn.* 155:1994-2004 ("Yelton et al."). According to the Examiner, it would have been prima facie obvious to one of ordinary skill in the art at the time the claimed invention was made to have produced a nucleic acid molecule that encodes a humanized antibody of Ferrara et al. by the methods of humanization of Adair et al. and Yelton et al. The Examiner

reasoned that one of ordinary skill in the art would have been motivated to and had a reasonable expectation of success to produce a nucleic acid encoding a humanized anti-VEGF antibody because: 1) Adair et al. teach a method for humanization of antibodies because "most Mabs are of rodent origin, they are naturally antigenic in humans and thus can give rise to an undesirable immune response termed the HAMA"; 2) Ferrara et al. teach the antibody can be humanized and the tumors from A4.6.1 treated animals were smaller than those tumors in mice treated with a control antibody; 3) Yelton et al. teach a method for affinity maturation of an antibody in order to "change the form, affinity, and potentially the specificity of Abs to optimize them for delivering a wide variety of therapeutic agents to tumor cells;" and 4) Ferrara et al. teach human VEGF and in view of Adair et al. it would be obvious to humanize the antibody for therapy. See Office Action at pages 5-6. Applicants respectfully traverse these rejections and their supporting remarks.

Claim 34 is directed to an isolated nucleic acid encoding a humanized variant of a parent anti-VEGF antibody which parent antibody comprises non-human variable domains. As presently claimed, all of the following elements are required for the humanized variant of the anti-VEGF antibody: a) it binds human VEGF with a $K_d$ value of no more than about 1 x $10^{-8}$M, said $K_d$ value being no more than about 6-fold of the $K_d$ value of the parent antibody; (b) it has an ED50 value of no more than about 5nM for inhibiting VEGF-induced proliferation of endothelial cells *in vitro*; and (c) it inhibits VEGF-induced angiogenesis *in vivo*, wherein 5mg/kg of the claimed humanized variant inhibits at least about 50% of tumor growth in an A673 *in vivo* tumor model. Accordingly, to render the present claims obvious over the cited references, it must be shown that one of ordinary skill in the art would have been motivated and had a reasonable expectation of success to produce an isolated nucleic acid encoding a humanized anti-VEGF antibody variant having all of the above recited properties. Applicants maintain that the teachings of the cited references, even if combined, would not have rendered obvious a humanized variant of a parent anti-VEGF antibody with the desired VEGF binding affinity as presently claimed, much less a humanized variant with the additionally claimed potencies both *in vitro* and *in vivo* (i.e., elements (b) and (c) of claim 34). In particular, applicants point out that it has been known in the art, and even

acknowledged in the cited references, that an antibody with higher binding affinities to its antigen does not necessarily exert desired efficacy when used in the context of cultured cells or *in vivo* therapeutic treatment.

Ferrara et al. describes monoclonal anti-VEGF antibodies having neutralizing and tumor inhibiting activities. Indeed, the murine anti-VEGF antibody A4.6.1 disclosed in WO 94/10202 was used to provide non-human CDRs for the humanized antibodies of the present invention. The cited reference also suggests in general making and using humanized anti-VEGF antibodies. However, as will be discussed in more detail below, by simply applying humanization methods of Adair et al. or Yelton et al. to the murine antibodies of Ferrara et al., one ordinary skill in the art would not have had a reasonable expectation of success to produce a humanized anti-VEGF antibody variant that not only minimizes the HAMA immunogenicity of a parent murine antibody, but also 1) binds hVEGF with a strong affinity that is no more than about 6-fold of the parent antibody; 2) inhibits VEGF-induced endothelial cell proliferation *in vitro* at a low dose (an ED50 value of no more than about 5nM); and 3) inhibits VEGF-induced angiogenesis *in vivo* with a high efficacy (inhibits at least about 50% of tumor growth in an A673 *in vivo* tumor model at 5mg/kg).

Adair et al. discloses a method of humanization combining CDR grafting with specific framework residue substitutions, based on studies of an anti-CD3 antibody OKT3. By examination of available X-ray structures, the reference has identified a number of framework residues which may have an effect on net antigen binding. Adair et al., at pages 20 and 38. Accordingly, a range of residues in the acceptor framework is proposed to be possible target(s) for donor residue substitutions in order to improve antigen binding (see, e.g., pages 16-19). Applicants submit that the teachings of Adair et al., at most, provide a general protocol for one of ordinary skill in the art to <u>try</u> different arrangements of residues within the heavy or light chain of a specific antibody. One would have to pick and choose which residues to be altered, if any, without sufficient guidance or expectation of success as to the desired binding affinity and biological activity.

Moreover, the results provided in Adair et al. itself showed that even those modified antibodies with increased binding affinity behaved differently in an unpredictable manner, when tested for their biological activities. In Example 5 (pages 61-64), for example, a number of murine anti-TGF-α mAbs were CDR-grafted (and FR residues swapped) according to the protocol used for OKT3 antibodies. Some of the resultant variants showed binding affinities similar to that of the murine or chimeric counterpart antibodies. But when assessed in an L929 cell competition assay in which the antibody functionally competes against the TNF receptor on L929 cells for binding to TNF in solution, theses antibodies failed to effectively compete with and block the TNF receptor-ligand interaction. Specifically, gL221/gH341, the humanized version of 61E71, was approximately 10% as active as murine 61E71 (page 61); the humanized version of hTNF3 binds well to TNF-α, but competes very poorly in the L929 assay (page 63); and the humanized 101.4 antibodies are at least an order of magnitude less able to compete for TNF against the TNF receptor on L929 cells (page 64). Thus, the Adair et al. reference itself showed that increased binding affinity of a humanized antibody as a result of using the methods taught therein can not be directly correlated to improved competitive activity in a cell assay. As such, humanizing a mouse anti-VEGF antibody of Ferrara et al. with the method of Adair et al. would not have produced an humanized variant of a parent anti-VEGF antibody that would have both the binding and the inhibition characteristics as claimed in the present invention.

50 Yelton et al. describes affinity maturation of a chimeric anti-carcinoma antibody, BR96, by codon-based mutagenesis. As stated clearly in the reference, the work carried out therein was focused on CDRs as opposed to framework residues; and mutagenesis was made within one single CDR at a time rather than multiple CDRs. Yelton et al. at page 2001, last paragraph on the left column. Similar to Adair et al., the selection of residues targeted for mutagenesis was based upon CDR loop assignments of BR96 defined by three dimensional modeling of the BR96 antibody variable fragment (right column on page 2001, citing Bajorath (1994) *Bioconjug. Chem.* 5:213).

BR96 is a mAb recognizing Lewis Y ($Le^y$)-related antigens expressed on the surface of many human carcinomas. The affinity mutants of BR96 were tested for their

binding affinities to either an enzyme conjugate of synthetic $Le^y$ tetrasaccharide ($sLe^y$) serving as an isolated antigen, or carcinoma cell lines expressing on their surface the $Le^y$ antigen. The results provided by Yelton et al. clearly show that the binding affinity to $sLe^y$ does not necessarily correlate with the binding affinity to tumor cells with $Le^y$ expressed and bound on their surface. For example, a mutant clone M4 was shown to bind $sLe^y$ with a 3-4 fold greater reactivity than M1 (another mutant), and an approximately 15-20 fold increase compared with the BR96 parent. Yet it did not show any improvement over M1 in binding to H3396 tumor cell membranes. Pages 1999-2000. Thus, antibody mutants generated according to Yelton et al. would not necessarily have desired binding affinity to its antigen in a native state, much less any therapeutic efficacy. Indeed, the authors of the references went on to postulate that increasing the affinity of an Ab (specific to a tumor antigen) may not bring a therapeutic advantage in treating tumors. Page 2002, bottom of the left column. Furthermore, the authors acknowledged that the optimal affinity may vary from Ab to Ab, depending upon the nature of the Ag and the targeted agent. Page 2002, last paragraph.

As such, humanizing a mouse anti-VEGF antibody of Ferrara et al. with the method of Yelton et al. would not have produced an humanized variant of a parent anti-VEGF antibody that would have both the binding and the inhibition characteristics as claimed in the present invention.

Thus, in light of the limited teachings of the cited references, the present invention as claimed would not have been obvious. Applicants respectfully request that the rejections under 35 U.S.C. §103 be reconsidered and withdrawn.

Applicants submit that the claims are now in condition for allowance. However, should there be any issues relating to this response or the subject application, applicants sincerely invite the Examiner to telephone the undersigned attorney to discuss and resolve them promptly. Applicants will be pleased to submit documents necessary to advance this application to issuance.

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required,

Applicants petition for any required relief including extension of time and authorizes the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to Deposit Account No. 07-0630. However, the Assistant Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

Respectfully submitted,
GENENTECH, INC.

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